Desflurane, compared to halothane, augments phenylephrine-induced contraction in isolated rat aorta smooth muscle

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Purpose: The mechanism responsible for the mediation of hypertension in response to increased desflurane levels is unclear. This study compared the effect of desflurane and halothane on phenylephrine (PE)-induced contraction in rat aorta ring and the effect of desflurane in the presence and absence of nitric oxide (NO) synthase activity.

Methods: Endothelium-free rat aorta rings were exposed serially to 10^{-7}M, 10^{-6}M and 10^{-5}M PE alone and subsequently in the presence of 2 MAC desflurane and halothane. Secondly, endothelium-free preparations were exposed to 10^{-6}M PE serially in the presence of 0, 1, 2 and 3 MAC desflurane and halothane. Thirdly, using an endothelium-intact preparation, the effect of desflurane on PE-induced contraction was examined, in the presence or absence of NG-nitro-L-arginine (L-NNA), an inhibitor of constitutive and inducible NO synthase.

Results: Contraction amplitudes secondary to 10^{-6} and 10^{-5}M PE in endothelium-free preparations were increased by 74% and 36% respectively ($P < 0.05$) in the presence of 2 MAC desflurane compared to controls. In endothelium- free preparations, contraction amplitudes secondary to 10^{-6}M PE were increased in the presence of 1 and 2 MAC desflurane by 32% and 18% respectively ($P < 0.05$) and reduced by 16% in the presence of 3 MAC halothane ($P < 0.05$). In endothelium-intact preparations an expected absolute increase in contraction amplitude occurred in the presence of L-NNA but the desflurane effect was detectable both in the presence and absence of L-NNA.

Conclusion: Our results suggest that desflurane may have a local vasoconstrictive effect independent of endothelium and NO synthase activity. The mechanism remains to be determined.

Objectif : Le mécanisme responsable de la médiation de l'hypertension en réponse à l'augmentation de desflurane n'est pas encore connu. La présente étude a comparé l'effet du desflurane et de l'halothane sur les contractions induites par la phényléphrine (PE), dans des anneaux d'aorte de rat, et l'effet du desflurane avec et sans activité de la synthétase de l'oxyde nitrique (NO).

Méthode : Des anneaux d'aorte de rat sans endothélium ont été exposés par série à de la PE de 10^{-7}M, 10^{-6}M et 10^{-5}M et, par la suite, à du desflurane et à de l'halothane à 2 CAM. Les préparations sans endothélium ont été ensuite exposées par série à de la PE à 10^{-6}M en présence de desflurane et d'halothane à 0, 1, 2 et 3 CAM. Enfin, en utilisant une préparation dont l'endothélium a été conservé intact, l'effet du desflurane sur la contraction induite par la PE a été examiné, en présence et en l'absence de NG-nitro-L-arginine (L-NNA), un inhibiteur de la synthétase de NO constitutive et inductible.

Résultats : L'amplitude des contractions secondaires à la présence de PE à 10^{-6} et à 10^{-5} dans des préparations sans endothélium a augmenté de 74 % et 36 %, respectivement ($P < 0,05$) avec du desflurane à 2 CAM, en comparaison avec les témoins. Dans les préparations sans endothélium, l'amplitude des contractions liées à de la PE à 10^{-6} s'est élevée en présence de desflurane à 1 et 2 CAM, de 32 % et de 18 %, respectivement ($P < 0,05$) et s'est abaissée de 16 % en présence d'halothane à 3 CAM ($P < 0,05$) . Dans les préparations à l'endothélium intact, une augmentation absolue présumée de l'amplitude des contractions est survenue en présence de L-NNA, mais l'effet du desflurane a été détectable en présence et en l'absence de L-NNA.

Conclusion : Nos résultats suggèrent que le desflurane pourrait avoir un effet vasoconstricteur local indépendant de l'endothélium et de l'activité de la synthétase de NO. Le mécanisme reste à déterminer.

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THE effect of volatile anesthetic agents on vascular smooth muscle contraction is complex and depends on the agonist used and the concentration of the inhalational agent.[1] In addition, inhalational agents affect nerve conduction and synaptic transmission by sympathetic nerve fibres which innervate arterial and arteriolar smooth muscle walls.[2] Norepinephrine is the main neurotransmitter at the vascular neuromuscular junction.[2]

Desflurane is a relatively new volatile anesthetic agent.[3] A rapid increase in desflurane levels is associated with an acute increase in sympathetic neural outflow[4,5] and with increased concentrations of circulating epinephrine and norepinephrine.[4] The site(s) responsible for the mediation of these responses to desflurane remain(s) unclear (Figure 1). Complete airway blockade, systemic lidocaine, and other forms of pharmacological prophylaxis, do not fully block the response.[6,7] In addition, the hemodynamic changes can be blunted independently of the sympathetic outflow response to rapidly increased desflurane concentration.[8] The interaction of desflurane locally with the action of α-agonists on vascular smooth muscle is unknown. The effect of halothane on vascular ring vasomotion responses as well as its interaction with endothelium-dependent vasodilatation has been well characterised.[9–12] There has been extensive research in the last decade on the interaction of anesthetic agents with the endothelium.[1,13,14] Inhalational agents inhibit endothelium-mediated vasodilation,[9–12] but they do alter endothelium-dependent relaxation in agent- specific ways.[15,16] The interaction of desflurane with the synthesis and action of endothelial nitric oxide (NO) remains unknown.

Therefore, the interaction of desflurane locally with the action of -agonists on vascular smooth muscle and its interaction with endothelial NO warrants further investigation. This study compared the effect of desflurane and halothane on the action of phenylephrine (PE) in a vascular smooth muscle preparation and, secondly, investigated the effect of NO on the interaction between desflurane and PE.

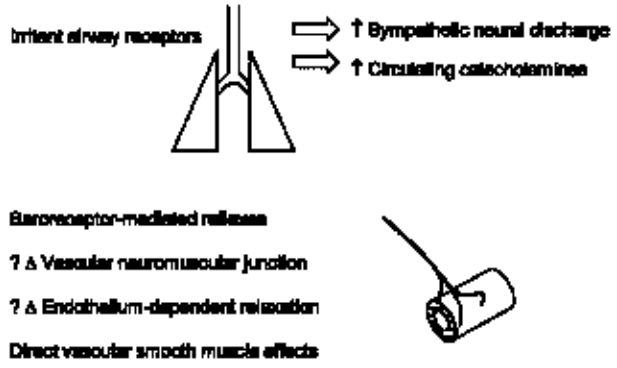

FIGURE 1 Possible sites and mechanisms underlying the hemodynamic effects of desflurane.

Materials and methods

Vascular ring preparation

Thirty-six male Wistar rats weighing between 300 and 400 g were studied. The animals were anesthetized with chloroform and then killed by guillotine. The descending thoracic aorta was dissected out in less than two minutes and suspended in a dissection tray of modified Krebs-Ringer bicarbonate solution at 37°C and oxygenated with a 95% O_2–5% CO_2 mixture maintaining a pH of 7.35–7.45. The Krebs-Ringer bicarbonate solution was freshly constituted for each experiment and composed of NaCl 113, KCl 5, $MgSO_4$ 0.9, $CaCl_2$ 1.4, $NaHPO_4$ 1.2, $NaHCO_3$ 25 and glucose 11.5 $mmol \cdot L^{-1}$. Two 3-mm rings of descending thoracic aorta were cut from the preparation in the dissection tray. For the first and second series of experiments, the endothelium was removed by rolling on a wet paper towel with the tip of a small surgical forceps for 15 sec and brushing of the luminal surface. The rings were suspended in a 10-ml water-jacketed organ bath maintained at 37°C, filled with Krebs-Ringer solution and continuously oxygenated with 95% O_2–5% CO_2 (Figure 2). One end of the ring was anchored to a fixed hook at the base of the bath while the opposite end was attached to an isometric force transducer (FT03C) via a hook and a silk thread. The

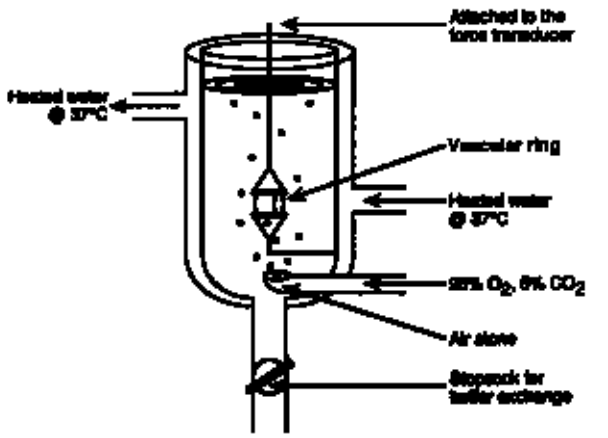

FIGURE 2 A diagrammatic representation of the water-jacketed organ bath. The vascular ring is suspended between the hook at the base of the bath and a silk thread attached to the isometric force transducer.

force transducer output was recorded by a Grass Model 7 polygraph, demonstrating changes in tension and by a computer to allow subsequent analysis of results.

Prior to each experiment calibration of the system was performed with 100 mg and 500 mg weights. Calibration was again confirmed at the end of each series of experiments. Each ring was then subjected to an optimal resting tension (1.5 g), established in a series of pilot experiments as the optimal passive tension that gave maximal active response to PE. This passive tension was maintained throughout the experiments. The muscle was then allowed to accommodate to the resting tension over a period of 60 min during which time the Krebs-Ringer bicarbonate solution was changed every 15 min. Absence of further relaxation with the addition of 10^{-9}M bradykinin confirmed adequate removal of the endothelium.

Experimental protocols

After this period and with evidence of complete accommodation to the resting tension (i.e., no evidence of further relaxation) a dose-response relationship for PE for each preparation was elicited (n=16). A contraction was obtained initially with 10^{-7}M PE followed by three to four washouts at ten-minute intervals with Krebs-Ringer bicarbonate solution. Further contractions were elicited with 10^{-6}M and 10^{-5}M PE using a similar washout protocol. The displacement was allowed to return to baseline before the next contraction was elicited. The response to PE was reproducible with repetitive administration of the agonist. A similar series of three contractions with 10^{-7}–10^{-5}M PE in the presence of 2 MAC desflurane or halothane (randomized) delivered by a vaporizer was then performed, following an initial equilibration period of 15 min. After a washout period of 30 min a control contraction in response to 10^{-6}M PE alone was elicited. After another washout period a further series of contractions in response to 10^{-7}–10^{-5}M PE in the presence of 2 MAC of the agent not previously used were performed. One MAC of desflurane in the rat is 5.7%[17] and 1 MAC of halothane is 0.82%.[18] The experiment was completed by another control contraction with 10^{-5}M PE following a washout period of 30 min. In order to evaluate the effect on basal tension, ten preparations were exposed to 2 MAC desflurane or halothane in the absence of PE for 30 min.

A second series (n=12) was performed to examine the effect of increasing concentrations of desflurane and halothane on PE-induced contractions. Based on the previous results, a concentration of 10^{-6}M PE was felt to be the optimum concentration to investigate the presence or absence of an inhalational agent effect. Following preparation as detailed above, a contraction was elicited with 10^{-6}M PE followed by three to four washouts at ten- minute intervals with physiological saline. The next contraction was elicited with 10^{-6}M PE 15 min following the addition of 1 MAC desflurane or halothane (randomized) and again followed by three to four washouts at ten-minute intervals. Further contractions were elicited in a similar fashion with 10^{-6}M PE following 15 min exposure to 2 MAC of the agent and subsequently to 3 MAC of the agent. It was ensured that the displacement returned to baseline after the washouts and before the next contraction was elicited. A control contraction in response to 10^{-6}M PE alone was elicited following a 30-min washout period. Another series of three contractions with 10^{-6}M PE, in the presence of 1, 2 and 3 MAC of the agent not previously used, were performed using a similar protocol. Finally, another control contraction in response to 10^{-6}M PE alone was performed following a 30-min washout period.

The third series (n=8) was performed to compare the interaction between desflurane and PE in the presence of intact endothelium and normal or inhibited NO synthase. The preparation and washout protocol was as above and contractions were elicited serially with 10^{-6}M PE in the presence of 0, 1, 2, 3 and 0 MAC desflurane. Both preparations were endothelium-intact and one was continuously exposed to 10^{-3}M L-NNA, a constitutive and inducible NO synthase inhibitor particularly effective in rat aorta.[19]

For all experiments, the amplitude of each contraction was measured and converted from millivolts to milligrams using the calibration values obtained for each preparation.

Agent concentrations

Desflurane and halothane were delivered from a vaporizer in the circuit delivering the O_2–CO_2 mixture to the bath. To determine the time of equilibration of desflurane and halothane, we used an agent analyzer (Datex Capnomac Ultima®, Helsinki, Finland) to measure the concentration of the agents in the bath above the Krebs-Ringer bicarbonate solution over 15 min. We found that desflurane concentrations were stable after three minutes and halothane concentrations stable after five minutes. We recorded the correlation between dialed agent concentrations and agent concentrations in the water bath at equilibrium with the Krebs-Ringer bicarbonate solution at five minutes.

Statistical analysis

Data were analyzed using multiple comparisons of repeated measurements corrected with the Bonferroni

TABLE I Dialed agent concentrations and the corresponding measured concentrations at equilibrium with the physiological saline in the organ bath

Desflurane Dialed (%)	*Measured (%)*	*Halothane Dialed (%)*	*Measured %*
5.7	4.72 ± 0.16	0.82	0.68 ± 0.05
11.4	9.89 ± 0.19	1.64	1.45 ± 0.06
17.1	15.82 ± 0.47	2.46	2.21 ± 0.12

Measurements taken at five minutes
Results expressed as mean % ± Standard Deviation
n=10

adjustment and repeated measures ANOVA with the Greenhouse-Geisser correction for multisample asphericity. Results are expressed as mean ± SEM. A *P* value <0.05 was considered statistically significant.

Results

Table I demonstrates the dialed agent concentrations and the corresponding measured inhalational agent concentrations at equilibrium with the physiological saline in the organ bath measured after five minutes. Figure 3 is an example of two contractions from the first series of experiments in the presence and absence of desflurane. The washouts are marked by artefacts and were commenced as soon as contraction amplitude reached a plateau. Neither desflurane nor halothane alone had a detectable effect on basal tension in the absence of PE.

The results of the first series are illustrated in Figure 4. PE induced contractions were greater in the presence of 2 MAC desflurane at PE concentrations of 10^{-6} and 10^{-5}M (428 ± 63 *vs* 246 ± 31 and 554 ± 69 *vs* 406 ± 56 mg, respectively, *P* <0.05). Contractions were similar in the presence of 2 MAC halothane compared to PE alone. ANOVA demonstrated a significant difference between the desflurane and both the halothane and control groups, but the absence of an interaction between PE concentration and presence of desflurane, suggesting a similar concentration-effect profile.

The results of the second series are illustrated in Figure 5. The presence of 1 and 2 MAC desflurane increased the amplitude of contractions (523 ± 75 *vs* 396 ± 49 mg and 469 ± 66 *vs* 396 ± 49 mg, *P* <0.05) but contractions were not significantly increased at 3 MAC. Halothane reduced the amplitude of contraction at 3 MAC (328 ± 41 *vs* 392 ± 45 mg, *P* <0.05). Contraction amplitudes at 1 MAC desflurane were greater than contraction amplitudes in the presence of 1 MAC halothane (523 ± 75 *vs* 385 ± 42 mg, *P* <0.05). ANOVA revealed a significant difference between the desflurane and halothane groups and a significant interaction between agent concentration and effect.

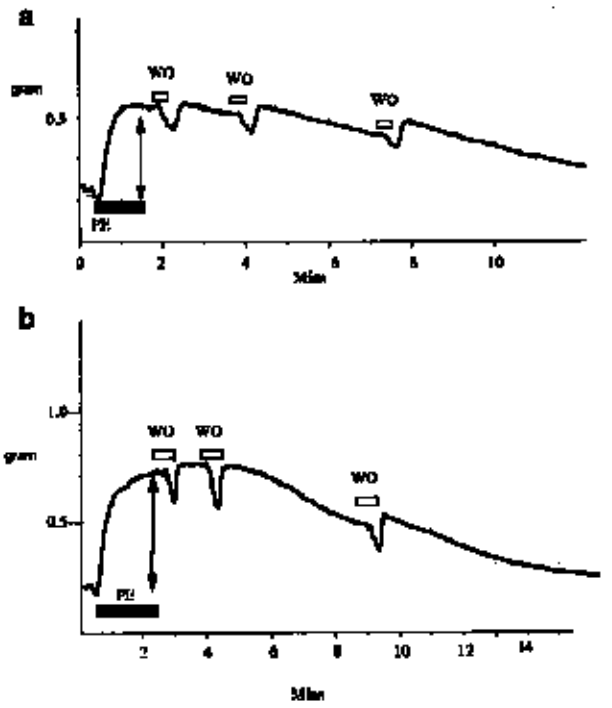

FIGURE 3 Representative recordings, from the first series of experiments, of isometric tension development in rat aorta smooth muscle exposed to phenylephrine (PE) alone (a) and PE in the presence of desflurane (b). An artefact marks the first washout (WO), performed as the contraction plateau phase begins. Washouts were repeated until isometric tension returned to baseline.

The results of the third series of experiments are illustrated in Figure 6 and Table II. Desflurane 1, 2 and 3 MAC significantly increased contraction amplitude in the presence of L-NNA and increased contraction amplitude at 2 and 3 MAC in the absence of L-NNA (Figure 6). All contraction amplitudes in the presence of an intact endothelium, including controls, were significantly lower than in the presence of L-NNA and also in the endothelium-free preparations in the previous series of experiments.

Discussion

These experiments demonstrate that 2 MAC desflurane, unlike halothane, increases contraction amplitude of endothelium-free vascular smooth muscle in response to PE 10^{-7}–10^{-5}M and demonstrate a maximal effect of desflurane at 1 MAC. Halothane demonstrates a significant inhibitory effect at 3 MAC. In endothelium-intact preparations, the pattern of the

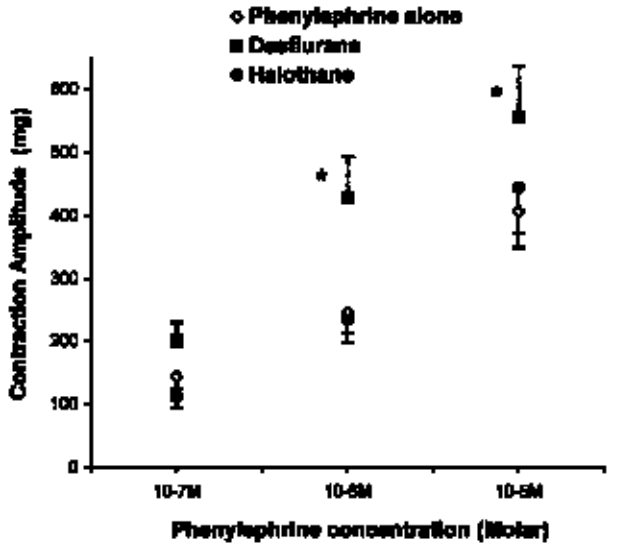

FIGURE 4 The effect of 2 MAC desflurane and halothane on the dose-response relationship of phenylephrine (PE)-induced contraction of rat aortic smooth muscle (n=16). Contraction amplitudes expressed in mg, means ± SEM. *=P < 0.05 compared to controls at equivalent PE concentration.

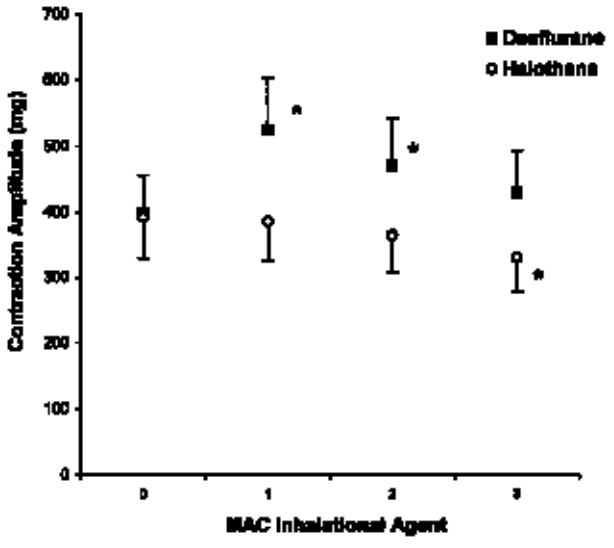

FIGURE 5 The effect of desflurane and halothane on rat aorta smooth muscle response to 10^{-6}M PE (n=12). Contraction amplitudes expressed in mg, means ± SEM. *=P <0.05 compared to control contractions to 10^{-6}M PE at 0 MAC.

desflurane effect is unaltered by the presence or absence of L-NNA which increases contraction amplitude as expected. Like other anesthetic agents, desflurane and halothane interact with vascular smooth muscle in three ways: firstly, via a direct effect on smooth muscle; secondly, via the interaction with endothelium-dependent relaxation; thirdly, via the interaction with neurotransmitter release at the level of nerve endings in the adventitia.

The direct smooth muscle effects of halothane and other inhalational agents have been clearly documented in tracheal and vascular smooth muscle.[20,21] Contraction is inhibited by at least three mechanisms: suppression of contractility independent of $[Ca^{++}]_i$ by interaction with contractile proteins, reduction of intracellular Ca^{++} release at low concentrations and by inhibition of voltage-and receptor-operated Ca^{++} channels at higher concentrations.[20,22–24] However, there is previous evidence that inhalational agents interact with different agonists in specific ways and interact selectively with particular vascular smooth muscle receptors. Halothane, but not isoflurane, for example, has been shown to have contractile effects in vascular tissues during specific conditions, possibly due to enhanced Ca^{++} release from intracellular Ca^{++} pools.[25–27]

The sites responsible for the mediation of the sympathetic activation and catecholamine release in response to rapidly increased desflurane concentration remain unclear (Figure 1).[6] Alfentanil has been shown to effectively blunt the hemodynamic changes associated with rapid increases in inspired desflurane concentration, without reducing activation of the sympathetic nervous system.[8] Therefore, the hypertension associated with a rapid increase in desflurane level may not be mediated solely by increased sympathetic outflow. The interaction of desflurane with PE, which we have demonstrated, suggests that a local interaction between desflurane and endogenous catecholamines or increased catecholamine release from adventitial nerve endings may occur. The fact that desflurane does not alter basal tension suggests that catecholamine release from nerve endings is not altered. However, further studies with measurement of catecholamine outflow or use of inhibitors of norepinephrine synthesis are required. Desflurane, like all inhalational agents, also has a direct smooth muscle inhibitory effect via a Ca^{++} mediated endothelium-independent pathway,[21] which may explain the lesser effect found at 3 MAC.

The interaction of inhalational agents with the vascular endothelium is complex.[13,14] Most studies suggest that inhalational agents inhibit vascular

TABLE II Amplitudes of aortic smooth muscle contraction in response to 10^{-6}M PE alone, or in the presence of L-NNA, with 0,1,2,3 and repeat 0 MAC desflurane

	0 MAC desflurane	*1 MAC desflurane*	*2 MAC desflurane*	*3 MAC desflurane*	*0 MAC desflurane*
10^{-3}M L-NNA + 10^{-6}M PE	292 ± 30.4	411 ± 37.1*	414 ± 30.1*	405 ± 49.5*	296 ± 28.6
10^{-6}M PE	182 ± 32.5	207 ± 39.7	247 ± 32.1*	254 ± 32.9*	189 ± 30.6

PE=phenylephrine; NG-nitro-L-Arginine=L-NNA; n=8
Results expressed in mg, mean ± SEM
*P < 0.05 compared to the initial control contractions at 0 MAC desflurane

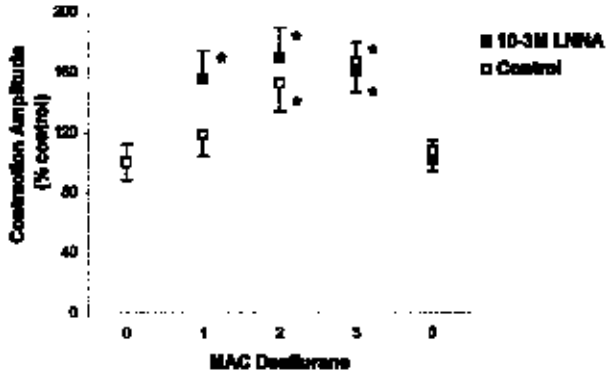

FIGURE 6 The effect of desflurane on rat aorta smooth muscle response to PE 10^{-6}M in the presence or absence of NG-nitro-L-arginine (L-NNA) (n=8). Contraction amplitudes expressed as percent of control contraction (at 0 MAC) for each preparation., means ± SEM. *=P <0.05 compared to corresponding control.

endothelium-dependent relaxation.[9–12, 28–30] There is evidence that isoflurane and halothane inhibit receptor- and Ca^{++}-activated NO synthase activity and may inhibit formation or release of NO.[11,31,32] Other studies have demonstrated inhibition of endothelium-dependent relaxation by sevoflurane, probably secondary to interaction of oxygen free radicals with NO, and direct endothelium-independent vasodilatory action.[16,33] Our results suggest that the absolute effect of desflurane on PE-induced contraction is less in the presence of intact endothelium and NO synthesis but that the mechanism is independent of NO as the overall pattern of the facilitatory effect is similar in the presence or absence of L-NNA.

Human *in vivo* studies have demonstrated that desflurane has a hemodynamic profile similar to isoflurane: tachycardia, hypotension and reduced systemic vascular resistance.[3 4] Studies on chronically instrumented animals have demonstrated that desflurane causes less direct vasodilatation than isoflurane.[3 5] *In vivo* studies have revealed a time factor in the sympathomimetic response to step increases in desflurane concentration.[4,5] *In vivo*, desflurane acts on vasculature with intact endothelium and autonomic innervation. In addition, the shorter time course of this *in vitro* study compared to *in vivo* studies, may allow demonstration of transient sympathomimetic effects not detected in long-term *in vivo* studies. It is possible that desflurane has a dual or biphasic effect, stimulating contraction initially or at lower concentrations and inhibiting contraction later or at higher concentrations. It is clear that there are several sites of action of desflurane on the contraction mechanism.

In summary, we have demonstrated that desflurane facilitates 10^{-7}–10^{-5}M PE-induced contraction of endothelium-free vascular smooth muscle and that the maximal facilitating effect of desflurane is at 1 MAC. In addition, the effect of desflurane on contraction amplitude is independent of the endothelium and the presence or absence of inhibitors of NO synthase. These data suggest that a local effect may partly mediate the hypertensive response to increased desflurane levels seen *in vivo*. Determination of the possible mechanism of this effect will require further investigation, in particular, investigation of catecholamine release, use of different agonists and pharmacological blockade of Ca^{++} channels and α and ß receptors.

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